The Osmotic and Chloride Regulative Capacities of Five Hawaiian Decapod Crustaceans[1]

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THE IONIC and osmotic regulative capacities of crustacean species have been described by a number of investigators (see reviews by Krogh, 1939; Robertson, 1953, 1960; Ramsay, 1954; Beadle, 1957; Lockwood, 1962, 1964; Potts and Parry, 1964). The most obvious and general conclusion which can be drawn from these investigations is that the aquatic crustaceans display varying degrees of responses to osmotic stress conditions. The animals' capacities to cope with the osmotic changes in the environment range from non-regulation or osmoconforming (the internal osmotic concentration maintained isosmotic to the environmental concentration) to hypo- and hyperosmotic regulation. The majority of the crustaceans appear to have the ability to regulate to some degree, either osmotically or ionically, within this wide range of regulatory capacities.

In the present investigation, the responses of five Hawaiian decapod crustaceans to varying sea water concentrations were studied. These five species were selected for study because of their ready availability in the shallow coastal waters off the island of Oahu. This investigation was undertaken to obtain basic information on osmoregulation in the common Hawaiian species which can be maintained in the laboratory and which may serve in further investigations on the mechanisms of salt and water balance in crustaceans.

MATERIALS AND METHODS

The five species of crabs were collected from various locations along the coastline of Oahu, Hawaii. All animals of the same species, however, were collected from the same general area. The semiterrestrial grapsid crab, *Metopograpsus messor* (Forskål) was collected from the mud flats of Kuliouou. This animal was usually found under rocks and debris which littered the flats. *Calappa hepatica* (Linnaeus), a member of the family Calappidae, was collected from the sand flats of Maunalua Bay. *Thalamita crenata* (Latrielle), *Podophthalmus vigil* (Fabricius), and *Portunus sanguinolentus* (Herbst) are all representatives of the family Portunidae. *T. crenata* is commonly found in brackish waters and was collected on the mud banks of Fort Kamehameha Reservation. *P. vigil* was trapped in 3 to 8 feet of water on the northeastern shore of Kaneohe Bay. *P. sanguinolentus* was collected on the reefs of Keehi Lagoon. After each collecting trip, the animals were brought back to the laboratory and kept in aerated, 100 per cent sea water (approximately 560 meq NaCl/1). Experimental salinities of 25, 50, and 75 per cent sea water were made by diluting 100 per cent sea water with appropriate amounts of tap water.

Animals were randomly selected without discrimination as to sex. Only animals in the intermolt stage were used for all of the experiments. For *C. hepatica* and the three portunids, the experimental media completely covered the animals. *M. messor,* however, was placed in water depths which permitted the animals to lift themselves above the water when they so desired. All animals were transferred directly into their test media from normal 100 per cent sea water.

At the end of a 24-hour exposure to the test media, the blood was analyzed for osmotic and chloride concentrations. Ten animals were used in each experimental group. Blood was obtained by puncturing the arthrodial membrane between the fourth and fifth thoracic appendages with a finely drawn capillary tube. The blood was expelled into a centrifuge tube and an equal volume of distilled water was added and thor-

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oughly mixed. Dilution was necessary to prevent total coagulation of the blood. The samples were centrifuged for 10 minutes at 10,000 rpm and the supernatant was analyzed for chloride and osmotic concentrations. The osmotic and chloride concentrations were determined on the Mechrolab vapor pressure osmometer and the Aminco-Cotlove chloride titrator, respectively. Osmotic concentrations were determined against known concentrations of NaCl and are expressed as milliequivalents of NaCl per liter (meq NaCl/l).

RESULTS

The blood osmotic concentrations of the five crab species in varying sea water concentrations are presented in Figure 1. As is apparent, *Calappa hepatica* has no ability to osmoregulate and is an osmoconformer. The blood osmotic concentration is essentially isosmotic with the ambient medium for all sea water concentrations tested. In 50 per cent sea water, mortality was approximately 70 per cent in 24 hours.

Both *Podophthalmus vigil* and *Portunus sanguinolentus* are able to maintain their blood osmotic concentrations slightly hyperosmotic in 75 and 50 per cent sea water. In 100 per cent sea water, *P. sanguinolentus* appears to hypo-osmoregulate. *P. vigil* is isosmotic to the ambient medium in 100 per cent sea water. Mortality was relatively high in 50 per cent sea water in both species.

Thalamita crenata is a good hyperosmoregulator in 50 and 75 per cent sea water and approaches isosmoticity in 100 per cent sea water. Mortality was relatively low in 50 per cent sea water. These animals do not survive in 25 per cent sea water.

Metopograpsus messor is an excellent osmoregulator, regulating hyperosmotically in sea water concentrations below 90 per cent, and hypoosmotically above this concentration. The blood osmotic concentration is maintained at a constant level in 25 to 100 per cent sea water. These animals can survive indefinitely in 25 per cent sea water under the conditions of this experiment.

The chloride concentrations in all crabs studied follow closely the changes in the blood osmotic concentrations when the animals are subjected to sea water concentrations of varying strengths (Fig. 2). The chloride accounts for nearly all the anionic contribution to the total osmotic concentration in the non-regulators and poor regulators with a lesser contribution of the chloride ion toward the total osmotic concentration in the regulators.

DISCUSSION

The results presented here were obtained from animals placed directly into artificially diluted sea water for 24 hours. Inasmuch as such abrupt changes in environmental conditions never occur under natural conditions, these results should not be interpreted as an indication of what occurs in nature but, rather, as a test of the adaptability and survivability of these animals under such adverse conditions. These data demonstrate, then, the ability of these animals to adjust to these abrupt changes over a relatively short period of time—an immediate adjustment which may be critical for the survival of the animal. Rapid and drastic changes in natural salinities, however, can occur in estuarine and semiterrestrial environments in which these animals are found. Strong dilutions of surface waters with consequent mass mortalities of marine organisms have been reported in Kaneohe Bay following torrential rains (van Weel and Christofferson, 1966). Under such conditions, survivability of an animal will depend upon its ability to adjust to, or to avoid, these drastically reduced salinities.

By regulating the variation of the osmotic concentration of its body fluid, an animal can adapt to an external environment in spite of a steep osmotic gradient. Two of the decapod species studied, *Metopograpsus messor* and *Thalamita crenata,* are able to maintain their blood osmotic concentrations hyperosmotic to dilute sea water concentrations. *Portunus sanguinolentus* and *Podophthalmus vigil* also hyperregulate, but poorly, in dilute media. *P. sanguinolentus* has some capacity to hyporegulate in 100 per cent sea water. Its capacity for some degree of regulation has also been suggested by George (1968), based on his studies of weight changes in the animals after transfer to dilute sea water. *Calappa hepatica* is an osmoconformer, unable to alter its blood osmotic concentration from that of the medium. Although data are presented for the last three species in 50 per cent sea water, it should be noted that their mortality is high in this dilute medium.

The extent to which an animal can survive variations in osmotic concentrations of its body fluid is limited to the range of osmotic concentrations in which the cells can still be functional. *C. hepatica, P. vigil,* and *P. sanguinolentus* can

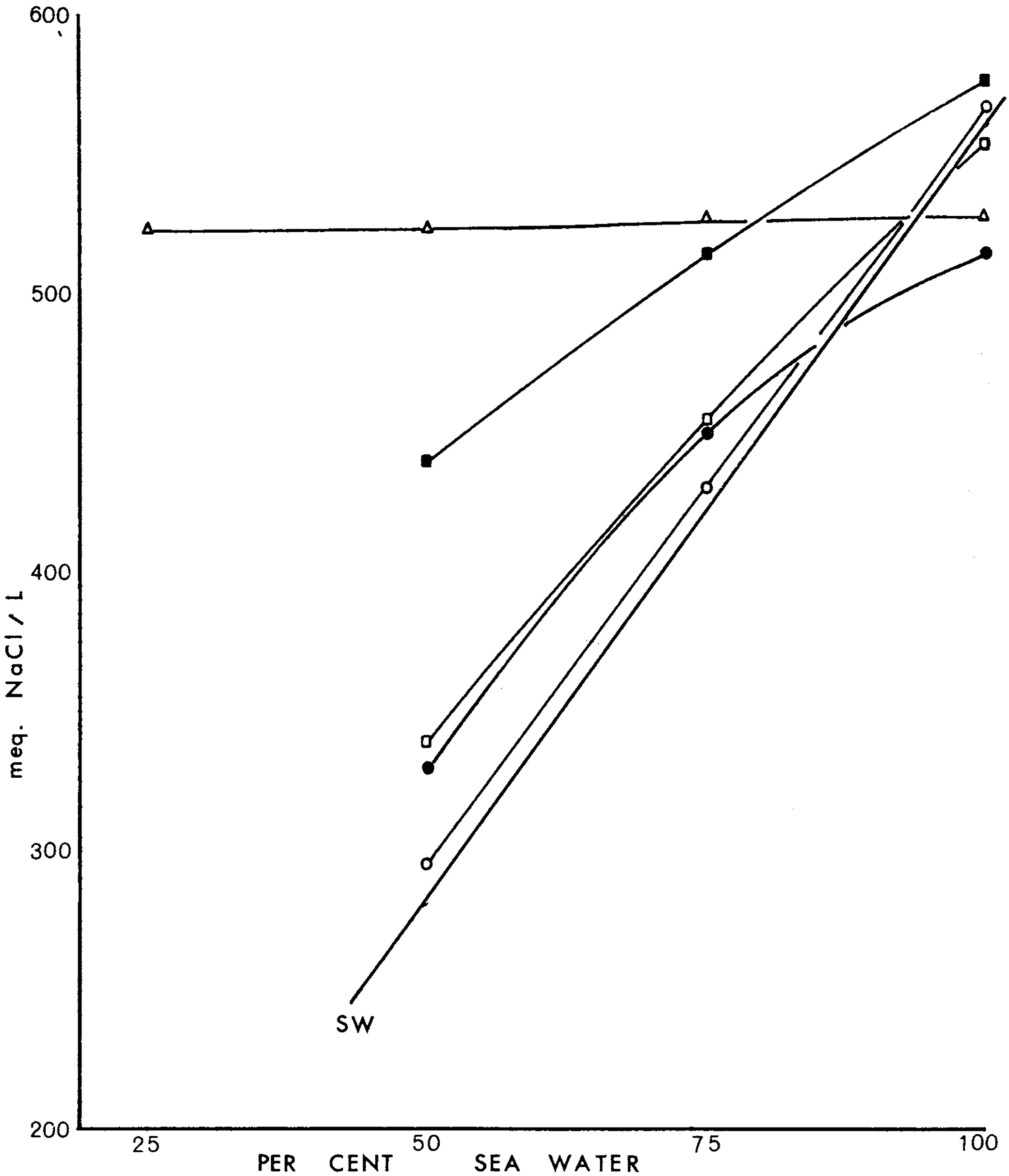

FIG. 1. Osmotic concentrations of the blood of five crustacean species placed in varying sea water concentrations for 24 hours. Each point represents the mean value for ten individuals. ○———○ *Calappa hepatica;* ●———● *Portunus sanguinolentus;* □———□ *Podophthalmus vigil;* ■———■ *Thalamita crenata;* △———△ *Metopograpsus messor.* Sw = sea water.

tolerate a fairly wide range of osmotic concentrations of their blood. No information is available on the osmotic concentrations of the cells in these varying sea water concentrations. It is possible that osmotic regulation may take place at the cellular level. Free amino acids or other organic compounds may contribute to the adjustment of cellular osmotic concentrations in these poor regulators, thus minimizing the osmotic effect, as has been proposed by Florkin and Schoffeniels (1965).

Obviously, most animals are best adapted to

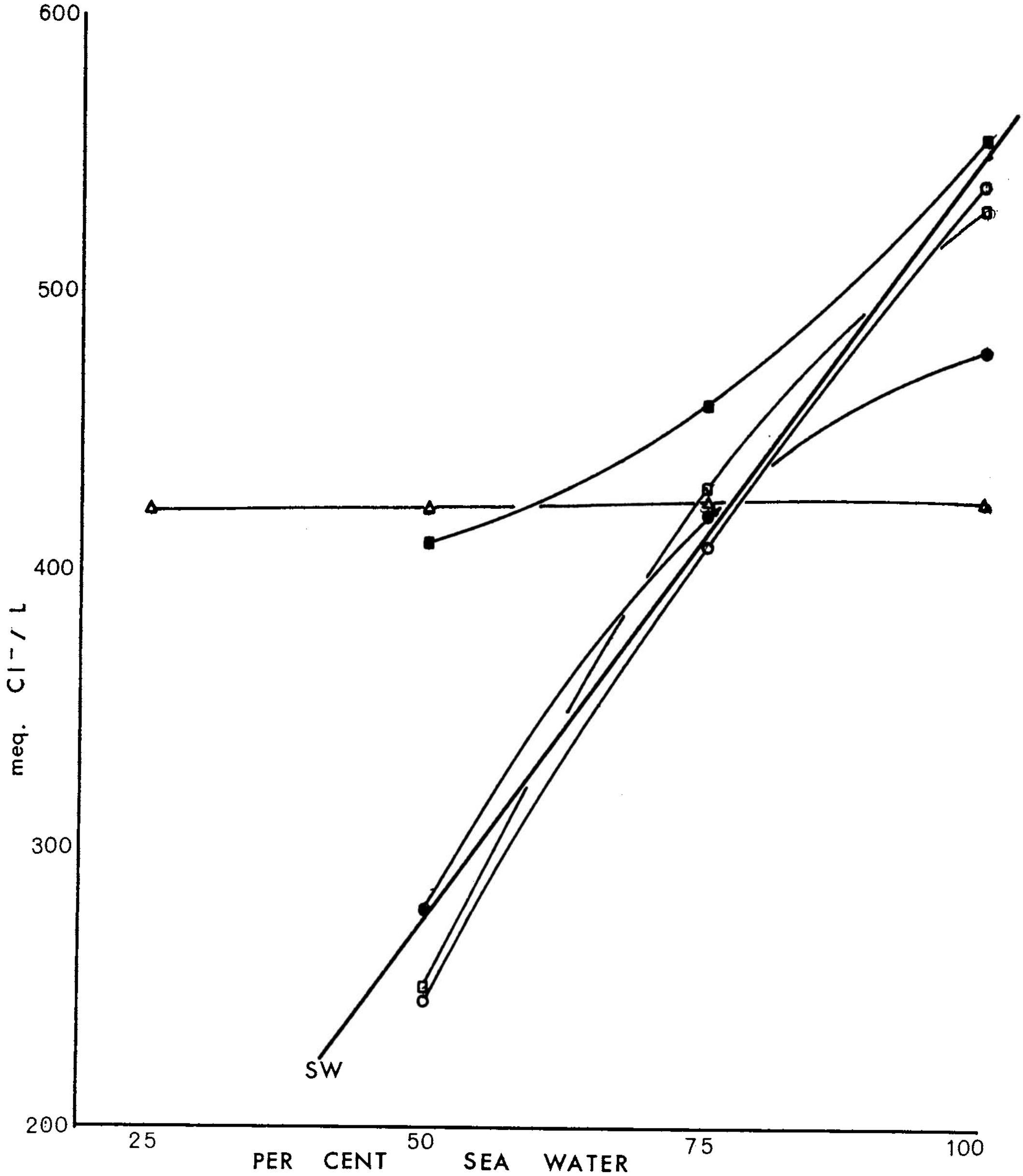

FIG. 2. Chloride concentration of the blood of five crustacean species placed in varying sea water concentrations for 24 hours. Each point represents the mean value of ten individuals. ○———○ *Calappa hepatica;* ●———● *Portunus sanguinolentus;* □———□ *Podophthalmus vigil;* ■———■ *Thalamita crenata;* △———△ *Metopograpsus messor.* Sw = sea water.

the particular environment in which they are normally found. The success of an animal to live in a variety of saline concentrations depends upon its ability to regulate osmotically. Indeed, *M. messor* and *T. crenata,* which are good regulators, are ubiquitous in distribution. *P. vigil* and *P. sanguinolentus,* which can tolerate 75 per cent sea water for extended periods, are found in the brackish waters of Kuliouou mud flats. These animals have also been reported in the Ala Wai Canal which drains fresh waters into the ocean.

The ability of an animal to maintain its internal osmotic concentration at a relatively constant level, as demonstrated by *M. messor,* may not be entirely due to physiological processes but also to behavioral factors. This can readily be seen if we compare the blood osmotic concentrations presented herein and those presented previously by Kato and Kamemoto (1968). *M. messor,* when permitted to submerge in the water or to lift itself out of the water, maintains a constant blood osmotic concentration over the range of 25 to 100 per cent sea water, "osmoregulating" in part by the regulation of its hydration or desiccation. Such behavioral regulation has been reported also by Gross (1964) for the terrestrial crabs *Coenobita* and *Cardisoma.* On the other hand, if the animals are completely submerged in aerated 25 per cent sea water (Kato and Kamemoto, 1968), the blood osmotic concentration drops to approximately 85 per cent of the concentrations reported here. Animals can live indefinitely under either condition without apparent ill effects.

Another interesting aspect of physiological-behavorial adaption by crabs to varying salinities has been reported by van Weel and Christofferson (1966) and van Weel and Correa (1966). In their electrophysiological studies on the activation of the osmoreceptors in various crabs, they showed that poor osmoregulating crabs (*Podophthalmus* and *Portunus*) were sensitive to a slight dilution of sea water while a good osmoregulator (*Thalamita*) was sensitive only to a greater dilution of sea water. They suggested that the poor osmoregulators, especially *Podophthalmus,* "osmoregulate" by avoiding dilute water, being able to perceive osmotic changes that take place in the environment.

The five decapod crustaceans studied here demonstrate a variety of osmoregulatory capacities. They should serve as excellent experimental animals for the study of the various mechanisms involved in the regulation of salt and water, and in the survival of these animals in a wide range of sea water concentrations.

SUMMARY

The osmotic and chloride regulatory capacities of five common Hawaiian decapod crustaceans were studied. *Metopograpsus messor* is an excellent osmoregulator, regulating both hypo- and hyperosmotically. *Thalamita* is a good hyperregulator in dilute sea water but an osmoconformer in 100 per cent sea water. *Portunus sanguinolentus* and *Podophthalmus vigil* can hyperregulate, but poorly, in dilute sea water. *Portunus sanguinolentus* hyporegulates in 100 per cent sea water. *Calappa hepatica* is an osmoconformer, unable to regulate its blood concentration in all salinities of its tolerance range.

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